

WE CLAIM:

1. A method for detecting the presence or activity of an analyte in a sample,

the method comprising:

5 forming a complex including first, second, and third members, where the first member is a probe, the second member is a mass label, and the third member is selected from the group consisting of the analyte, a compound that specifically binds to the analyte, and a product formed by the analyte, wherein no significant binding occurs involving the first and second members in the absence of the third member;

10 measuring a property of the probe that is sensitive to the size of the complex; and

correlating the property with the presence or activity of the analyte in the sample.

2. The method of claim 1, wherein the third member is the analyte.

15 3. The method of claim 1, wherein the third member is a receptor for the

analyte.

4. The method of claim 1, wherein the third member is an enzyme.

20 5. The method of claim 1, wherein the probe is photoluminescent.

6. The method of claim 5, wherein the step of measuring a property of the probe includes the step of detecting a change in polarization.

7. The method of claim 5, wherein the photoluminescence lifetime of the probe is greater than the rotational correlation time of the unbound probe and less than the rotational correlation time of the complex formed by binding of the first, second, and third members.

8. The method of claim 1, wherein the probe binds to the third member noncovalently.

9. The method of claim 8, wherein the probe includes at least one of an immunological binding partner of the third member and a particulate.

10. The method of claim 1, wherein the mass label includes at least one of an immunological binding partner of the third member and a particulate.

11. The method of claim 1, wherein the mass label is capable of specifically binding to more than one third member.

12. The method of claim 1, the mass label being a first mass label, further comprising a second mass label capable of specifically binding to at least one third member, the complex formed by binding of the probe to the third member, and the mass label, but not to the probe alone.

5

13. The method of claim 12, wherein the second mass label is capable of specifically binding to at least two first mass labels, so that the second mass label may form crosslinks between third members.

10
11
12
13
14
15

14. The method of claim 12, wherein the second mass label includes at least one of the following: avidin, biotin, lectin, sugar, and an immunological binding partner.

15. The method of claim 1 further comprising the step of selecting the probe and mass label such that the average number of mass labels bound to third members exceeds the average number of probes bound to third members.

16. The method of claim 1, wherein the property of the probe is related to a rotational diffusion coefficient of the probe.

17. The method of claim 16, wherein the property of the probe is measured using a technique selected from the group consisting of polarization, light scattering, and magnetic resonance.

5 18. The method of claim 1, wherein the property of the probe is related to the translational diffusion coefficient of the probe.

10 19. The method of claim 1, the sample being a first sample, further comprising repeating with a second sample the steps of forming a complex, measuring a property of the probe, and correlating the property with the presence or activity of the analyte.

15 20. The method of claim 19 further comprising the step of comparing the amounts or activity of analyte in the first and second samples.

20 21. The method of claim 1, wherein the step of correlating the property with the presence or activity of analyte in the sample includes the step of quantifying the amount of analyte in the sample.

25 22. The method of claim 1 further comprising the step of correlating the presence of analyte with the presence or activity of another compound.

23. The method of claim 22, wherein the other compound is an enzyme.

24. The method of claim 1, wherein the step of correlating the property with the presence of analyte includes the step of comparing the property measured in the sample
5 with the property measured in the absence of analyte.

25. The method of claim 1 further comprising the step of forming a complex includes the step of selecting the probe and mass label.

10 26. The method of claim 1, wherein the step of forming a complex includes the step of contacting the sample with the probe and mass label.

27. A kit for detecting the presence or activity of an analyte in a sample, the kit comprising:

a probe capable of specifically binding to a member, where the member is selected from the group consisting of the analyte, a compound that specifically binds to the
5 analyte, and a product formed by the analyte; and

a mass label capable of specifically binding to the member or to a complex formed by binding of the probe to the member, but not to the probe alone;

wherein a measurable property of the probe is sensitive to the size of the complex formed by binding of the probe, member, and mass label.

10 28. The kit of claim 27, wherein the probe is photoluminescent.

15 29. The kit of claim 28, wherein the photoluminescence lifetime of the probe is greater than the rotational correlation time of the unbound probe and less than the rotational correlation time of the complex formed by binding of the probe, member, and mass label.

30. The kit of claim 27, wherein the probe binds to the member noncovalently.

20 31. The kit of claim 30, wherein the probe includes at least one of an immunological binding partner of the member and a particulate.

32. The kit of claim 27, wherein the mass label includes at least one of an immunological binding partner of the member and a particulate.

33. The kit of claim 27, wherein the mass label is capable of specifically 5 binding to more than one member.

34. The kit of claim 27, the mass label being a first mass label, further comprising a second mass label capable of specifically binding to at least one of the member, the complex formed by binding of the probe to the member, and the first mass label, but not to the probe alone.

35. The kit of claim 34, wherein the second mass label is capable of specifically binding to at least two first mass labels, so that the second mass label may form crosslinks between members.

36. The kit of claim 34, wherein the second mass label includes at least one of the following: avidin, biotin, lectin, sugar, and an immunological binding partner.

37. The kit of claim 27, wherein the probe is not normally present in the 20 sample.

38. The kit of claim 27, wherein the mass label is not normally present in the sample.

39. The kit of claim 27, wherein the property of the probe is related to a
5 rotational diffusion coefficient of the probe.

40. The kit of claim 39, wherein the property may be measured using a technique selected from the group consisting of polarization, light scattering, and magnetic resonance.

41. The kit of claim 27, wherein the property of the probe is related to the translational diffusion coefficient of the probe.

42. A method of performing a resonance energy transfer assay, the method comprising:

providing first and second members of a donor/acceptor pair, the pair being capable of resonance energy transfer;

binding the first member to a binding partner;

20 permitting the first member to be diffusional mobile relative to the binding partner while it is bound to the binding partner; and

detecting a change in proximity between the first and second member.

43. The method of claim 42, wherein the first member is associated with a first binding partner and the second member is associated with a second binding partner, and wherein the change in proximity between the first and second members is due to binding between the first and second binding partners.

5

44. The method of claim 42, wherein the first and second members are associated with a common substrate, and wherein the change in proximity between the first and second members is due to cleavage of the common substrate.

10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95

45. The method of claim 43, wherein the common substrate is a protein, and wherein the cleavage is effected by a protease.

46. The method of claim 43, wherein the common substrate is a nucleic acid, and wherein the cleavage is effected by a nuclease.

15

47. The method of claim 42, wherein the first member is diffusional mobile across a surface.

48. The method of claim 47, wherein the surface is selected from the group consisting of a planar lipid bilayer, a liposome, and a cell membrane.

49. The method of claim 47, wherein the surface includes a plurality of first members.

50. The method of claim 42, wherein the first member is bound to the binding partner by a tether, the tether being sufficiently flexible that energy transfer between the first and second members is diffusionaly enhanced.

51. The method of claim 42, wherein the step of detecting a change in proximity includes the steps of:

10 illuminating the members, so that the donor is excited;
detecting light emitted from at least one of the members; and
calculating the amount of energy transfer between the donor and acceptor based on
the emitted light.

15 52. The method of claim 51, wherein the detected light is emitted by the donor.

53. The method of claim 51, wherein the detected light is emitted by the acceptor.

20 54. The method of claim 42, wherein the photoluminescence lifetime of the donor exceeds the photoluminescence lifetime of the acceptor.

55. The method of claim 42 further comprising the step of correlating the change in proximity with the presence of an analyte in a sample.

56. The method of claim 55, the sample being a first sample, further comprising repeating the steps of providing, binding, permitting, and detecting using a second donor/acceptor pair, and correlating the change in proximity with the presence of analyte in a second sample.

10 57. The method of claim 42 further comprising the steps of binding the second member to a second binding partner, and permitting the second member to be diffusionaly mobile relative to the second binding partner while it is bound to the second binding partner.

15 58. A composition of matter comprising:
a particulate, and
a luminophore associated with the particulate,
wherein the size of the particulate and the lifetime of the luminophore are selected so that depolarization of the luminophore is detectable in a polarization assay.

59. The composition of claim 58, wherein the particulate is selected from the group consisting of a macromolecule, a dendrimer, a glass bead, a latex bead, a polyacrylnitrile bead, and a liposome.

5 60. The composition of claim 58, wherein the luminophore is encapsulated in the particulate.

61. The composition of claim 58, wherein the luminophore is covalently bound to the particulate.

10 62. The composition of claim 58, wherein the luminophore is entrapped in the particulate.

15 63. The composition of claim 58, wherein the luminophore is a metal-ligand complex.

64. The composition of claim 58, wherein the luminophore is coupled to the particulate so that the luminophore retains a fixed orientation relative to the particulate.

20 65. The composition of claim 58, wherein the luminophore is Ru-trix-bathophenanthroline.

66. The composition of claim 58, wherein the size of the particulate and the lifetime of the luminophore are selected so that luminescence emitted from free particulate bound to luminophore in a luminescence polarization assay is substantially unpolarized.

5

67. The composition of claim 58, wherein the particulate is labeled with at least one ligand.

68. The composition of claim 58, wherein the particulate is labeled with multiple ligands.

69. A method of detecting the presence or activity of a molecule of interest in a sample, the method comprising:

providing a particulate associated with a luminophore wherein the lifetime of the luminophore is long enough relative to the size of the particulate so that luminescence emitted from the luminophore is significantly depolarized,

contacting the particulate and luminophore with a sample containing the molecule of interest,

detecting a change in polarization of luminescence emitted by the luminophore,

20 and

correlating the change in polarization with a property of the molecule of interest.

70. The method of claim 69 further comprising the step of selecting a size of the particulate and a lifetime of the luminophore so that luminescence emitted from free particulate bound to luminophore in a luminescence polarization assay is substantially unpolarized.

5

71. The method of claim 69 further comprising the step of encapsulating the luminophore in the particulate.

72. The method of claim 69 further comprising the step of entrapping the luminophore in the particulate.

73. The method of claim 69 further comprising the step of covalently binding the luminophore to the particulate.

74. The method of claim 69 further comprising the step of labeling the particulate with at least one ligand.

75. The method of claim 69 further comprising the step of agglutinating multiple particulates in the presence of the molecule of interest.

20

76. A method of performing a resonance energy transfer assay, the method comprising:

providing first and second members of a resonance energy transfer pair, wherein the first member is associated with a particulate,

5 contacting a sample with the resonance energy transfer pair under conditions such that proximity of the first member relative to the second member indicates a property of a molecule of interest.

77. The method of claim 76, wherein the providing step includes the step of associating the first member with a particulate selected from the group consisting of a macromolecule, a dendrimer, a glass bead, a latex bead, a polyacrylnitrile bead, and a liposome.

78. The method of claim 76, wherein the providing step includes the step of 15 encapsulating the first member in the particulate.

79. The method of claim 76, wherein the providing step includes the step of entrapping the first member in the particulate.

20 80. The method of claim 76, wherein the providing step includes the step of covalently binding the first member with the particulate.

81. The method of claim 76, wherein the providing step includes the step of associating plural first members with a single particle.

82. The method of claim 76, wherein the property of the molecule of interest
5 may be quantitative presence, qualitative presence, or activity.